Rotation Axes for Analysis of Gravity Effects on Plant Organs

Charles J. Lyon
Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

Received March 10, 1967.

Summary. Epinastic curvatures of branches of Coleus blumei Benth. and the growth pattern of wheat (Triticum aestivum L.) seedlings on clinostats were used for bioassay of rotation methods for preventing growth responses to gravity. Tumbling a plant end over end was found to be just as effective as rotation about its horizontal axis. The results support the reliability of data from experiments in which an entire plant is rotated about a single horizontal line with only part or none of its immature tissues in horizontal orientation to gravity.

The clinostat was devised as a tool in the early studies of geotropism (18). The name suggests its use with the rotating axis of a plant inclined at some angle but such experiments were uncommon and relatively unproductive. In tests of growth curvatures after geotropic stimulation at various angles (5) and in measurements of presentation time and reaction time (2, 3, 16, 17), the axis of rotation was set at 90° with the vertical to avoid further stimulation by gravity. In subsequent studies of geotropism and plant morphology on the basis of internal growth factors, most tissues have likewise been rotated in vertical planes. The horizontal clinostat is therefore descriptive of the usual orientation of its rotation axis.

Precision in maintaining a horizontal position has sometimes been emphasized as essential for the derivation of reliable data (6). In the same analysis of methods it was proposed that the supports of certain test plants should also be kept horizontal while Newcombe (15) concluded that an erect, branched plant must be rotated with the stem at right angles to the horizontal. Simultaneous rotation about 2 axes has also been tested as a possible improvement in eliminating the effects of gravity (8).

When epinastic growth curvatures were first recognized as such in the immature leaves and branches (4) of a plant with its foliage revolving about the horizontal axis of the plant, it was clear that the tendency to epinasty had been revealed only because the geotropic responses of the lateral organs had been prevented. Yet these organs had not been rotated about their axes, and their axes had not remained perpendicular to the lines of gravitational force. Only the plant's main stem had been horizontal and its tissues were not linear. In his review of methods for studying geotropism, Larsen (9, p 60) accepted such demonstrations of epinastic curvatures as valid but did not explain how the gravitational stimulus had been rendered ineffective.

In the same way a curvature in the growth zone of the rotated horizontal axis of a plant like Coleus continues to develop asymmetrically at some angle with the axis (10, 13) after an early curvature has placed the youngest internodes in a position corresponding to that of a petiole or branch. The geotropic responses of the immature tissues are eliminated by rotation without horizontal orientation of the growth zone. The axial curvatures were shown to be due to uneven distribution of IAA in the absence of a gravity vector (13). The same imbalance probably produced many of the curvatures in the rotated Xanthium stems as reported by Hoshizaki and Hammer (7).

The continual rotation of a growth zone about a horizontal line seems to be effective in preventing a geotropic curvature because the receptor tissue is turned to a new orientation with gravity before the chain of events of the presentation period has been completed. The constant interruption in reaction to a gravity vector prevents the multilateral stimulus from being effective for stimulation of unbalanced growth.

If this continual change in orientation of receptors is the cause of the failure to respond to the stimulus, it is not essential that the plant's axis of symmetry be the axis of rotation. The same rotation effect should be produced by tumbling any erect plant end over end in vertical planes about an axis outside the regions of receptor tissue. Possible imbalance in illumination or other factors that might introduce confusing growth changes in the test material can be avoided with experiments in darkness at constant temperature.

---

1 Supported by the National Aeronautics and Space Administration through research grant NSG-231 and research contract NAS2-1558.
Material and Methods

**Potted Plants.** I have used the growth curvatures of branch epinasty (11) in *Coleus blumei* Benth. for bioassay of comparative effects of different rotation axes on the lateral organs of a shoot. Plants from an established clone were cultured for 2 strong branches each and selected in groups of 10 for branch length of 10 to 20 cm. The 20 branches were defoliated, the tips cut back to the first firm node, and a cap of 1% indoleacetic acid (IAA) in lanolin applied to the freshly cut end of each branch. The initial angular position of each branch was then recorded on paper by shadowgraph tracings as previously described (11) for study of the epinasty.

The 10 plants were attached in 2 sets of 5 each (13, figs 1-2) to a multiple clinostat that turned at 6 rpm, with the stems of the plants either parallel or at right angles to the horizontal axis of revolution. The potted plants in the second position were held in special cradles as they were tumbled end over end through a circular path about 15 cm from the clinostat axis, with the pairs of branches oriented either in the plane of rotation or normal to this plane.

Measurements of epinastic curvatures in the 20 branches of each test were obtained from changes in the orientation of their youngest internode after 12 hours of rotation in darkness at 24.5 ± 0.5°. The second set of branch position tracings was made on the same sheet as the first, with the shadows of fixed reference stakes superimposed. The angle between tangents drawn to the initial and terminal position tracings was used as a measure of the imbalance of growth due to absence of a gravity effect on lateral transport of IAA (12) within the branch.

After the plants had been left erect to gravity in the same dark room for 24 hours to permit growth adjustments of the branches to their plagiotropic positions, the 10 pairs of branches were used for a second test, either with a change of orientation or with the same positions of the branches for duplicate measurements. In all cases the number of 12-hour periods of rotated growth with epinastic curvatures was divided equally between day and night hours for any 1 position, since the growth rates of the branches were always greater during the period between 8 P.M. and 8 A.M.

**Germinating Seeds.** The orientation of wheat seedling organs as they develop on clinostats was used to compare the effect of rotation about the seedling axis (in horizontal position) with that of tumbling the seed (fig 1) as it germinates with the coleoptile and roots growing in moist air. The special apparatus and cultural method for growing such seedlings in sets of 15 within a closed cylinder were described recently (14) along with the method for measuring the orientation of each organ in terms of its angular position as recorded by photographs of face and side views.

The rotation rate was 1 rpm for both rotation systems. Control experiments with the seedlings growing erect to gravity on a turntable tested possible effects of the same rotation rate on the orientation of axial and lateral tissues.

The wheat seedling experiments were carried out in the dark constant temperature room used for the *Coleus* work but with the temperature reduced to 20 ± 0.5°. The relative humidity outside the closed cylinders (fig 1) was held at 85% so that the orientation of the roots would remain unchanged when the seedlings were removed for photography after 79 hours of growth.

**Experimental Results and Discussion**

**Coleus Branches.** The results from rotating *Coleus* branches in 3 directions are shown in table I. The effect in all cases was to produce similar epinastic curvatures in the younger internodes. There are no significant differences in the mean values for the degrees of curvature. The relatively large standard errors of the means are due primarily to the effect of diurnal variations in growth rates on the curvatures that could be formed within a 12-hour period of growth.

The tumbling motion, with the branch tissues moving either in the rotation plane of the tumbling or oriented normal to it, was as effective in inducing epinastic curvatures as swinging the branches about the horizontal stem to which they were attached. Since the axis of rotation of this stem was outside the growth zones of the branches only by a shorter distance than was the axis for the 2 rotation systems called tumbling, there were no real differences in the 3 treatments of the growth zones. In no case was the centrifugal force large enough to be an effective stimulus. The distribution of growth hormone must be equally unbalanced (11) in each of the systems of rotation.

From this it appears possible to use a variety of uniform, cyclic disturbances in the vectorial action of gravity on receptor tissues held at various angles in the lateral organs. However, for practical reasons of simple design and ease of attaching an entire plant firmly to a clinostat plate, the traditional form with the main stem approximately horizontal will usually be preferred. Crocker, Zimmerman and Hitchcock (1) used a tumbler form for a few tests of leaf epinasty in tomatoes

Table I. **Epinastic Curvatures of Coleus Branches on Clinostats with Different Axes of Rotation**

<table>
<thead>
<tr>
<th>Rotation method</th>
<th>No of branches</th>
<th>Mean curvature in 12 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem horizontal</td>
<td>160</td>
<td>82.7 ± 3.3*</td>
</tr>
<tr>
<td>Rotation tumble</td>
<td>160</td>
<td>80.1 ± 3.7*</td>
</tr>
<tr>
<td>Normal tumble</td>
<td>80</td>
<td>76.2 ± 5.6*</td>
</tr>
</tbody>
</table>

Downloaded from on June 26, 2017 - Published by www.plantphysiol.org
Copyright © 1967 American Society of Plant Biologists. All rights reserved.
Fig. 1. Clinostat wheel with 2 cylinders of wheat seedlings grown during tumbling in plane of paper for 79 hours at 1 rph.
but rotated most of their plants with the stems horizontal.

Wheat Seedlings. The 2 horizontal axes of rotation for the wheat seedling tests provided a comparison of tumbling an entire axis versus simple rotation of axial tissues in horizontal position. The effects on the orientation of the developing roots and coleoptiles appear in the data for mean angular positions as recorded in table II. There are no significant differences between the positions of corresponding organs as they develop under the 2 forms of rotation. The mean curvature in a coleoptile is simultaneously lateral to the seed axis and outward from the endosperm to a greater degree than in an erect seedling. The primary root shows equally greater deviations from the 180° position (in face view) than it does when the force of gravity acts downward along the seedling axis. The side view angles are also equally greater on the 2 kinds of clinostats for both primary and lateral roots.

The development of the same epinastic curvatures in the lateral roots (as recorded in face view) under the 2 clinostat methods corresponds to the production of equal degrees of mean curvatures in Coleus branches through their rotation by the normal tumble and stem horizontal methods (cf. table I). Elimination of geotropic responses by some system of rotation about a horizontal line applies alike to seedlings and established plants. Lateral roots of barley have been found (8) to exhibit strong epinastic growth when the seeds were germinated for 3 days on a 2-axis clinostat. There appears to be no difference or advantage derived from use of a second axis.

Possible effects of the slow rotation itself on the orientation of plant organs are shown by the data of table II to be absent in wheat seedlings erect to gravity. There are no significant differences in the mean positions of their roots and coleoptiles between plants grown stationary or on a turntable at 1 rph. Rotation in any plane at a rate high enough to introduce vibrations or torsional stresses within growing tissues might act indirectly to influence the direction or form of growth of an organ, but motion alone proved to have no measurable effect on the growth pattern of a wheat seedling. Motion is an essential feature of an acceptable clinostat because the changes in orientation to gravity are rotary and uniform about a horizontal line.

**Table II. Mean Orientation Angles of Wheat Seedling Organs in Moist Air**

Germination for 79 hours at 20° in darkness and closed cylinders.

<table>
<thead>
<tr>
<th>Position</th>
<th>Seed. axis</th>
<th>Seed. no</th>
<th>Coleoptile Face view</th>
<th>Prim. root Face view</th>
<th>Left root Face view</th>
<th>Right root Face view</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinostat</td>
<td>Hori.</td>
<td>84</td>
<td>10.7 ± 1.1 16.1 ± 1.4</td>
<td>46.1 ± 6.6 55.7 ± 5.2</td>
<td>289.0 ± 2.4 133.2 ± 5.2</td>
<td>79.8 ± 2.7 112.0 ± 6.8</td>
</tr>
<tr>
<td></td>
<td>Tumb.</td>
<td>86</td>
<td>8.9 ± 0.9 11.6 ± 0.8</td>
<td>61.8 ± 5.9 66.4 ± 4.5</td>
<td>287.0 ± 1.6 133.0 ± 4.0</td>
<td>82.8 ± 1.9 108.0 ± 6.0</td>
</tr>
<tr>
<td>Erect</td>
<td>Stat.</td>
<td>44</td>
<td>2.6 ± 0.6 2.5 ± 0.4</td>
<td>179.3 ± 21.1 19.0 ± 1.9</td>
<td>245.7 ± 1.6 21.4 ± 2.1</td>
<td>114.7 ± 1.4 24.2 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>Turn.</td>
<td>42</td>
<td>3.4 ± 0.5 4.0 ± 0.7</td>
<td>181.7 ± 1.9 17.0 ± 1.8</td>
<td>241.6 ± 1.3 22.0 ± 2.5</td>
<td>120.6 ± 1.6 18.2 ± 2.5</td>
</tr>
</tbody>
</table>

* Mean angular deviation of primary root from 180° position. These deviations for erect positions below were:
  Stationary 9.7 ± 1.4 and turntable 8.6 ± 1.4
** Angle measures the mean curvature from 0°, left or right.

**Literature Cited**